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Latent period and viability of *Puccinia jaceae* var. *solstitialis* urediniospores: Implications for biological control of yellow starthistle

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Abstract

Since the introduction of *Puccinia jaceae* var. *solstitialis* into California in 2003, multiple urediniospore generations have been observed, along with teliospores and pycnia, on yellow starthistle (*Centaurea solstitialis*). A field and laboratory study of urediniospore viability was conducted to determine the potential of using infected plant material for redistribution of inoculum into new areas. To better understand the life history of *P. j. solstitialis*, urediniospore viability was measured during the summer and fall when the host plant is usually senescent. Latent period (time from inoculation to pustule eruption) after field and laboratory inoculations was measured in order to determine the potential number of urediniospore generations that occur per year. Viability of urediniospores stored in the laboratory gradually declined over a period of 10 weeks; spore hydration increased germination. Based on the results obtained in this study, introductions of harvested infected plant material would have to be done quickly to offset losses in urediniospore viability. Urediniospore viability in the field decreased exponentially 86% near the coast and 97% in the Central Valley three weeks after production, and was negligible (0–0.2%) thereafter. Latent period ranged from four to five weeks at cool winter temperatures to two to three weeks at warm summer temperatures in both the laboratory and field. Results of this study suggest that urediniospores are not likely to remain viable through the summer and fall dry season. Teliospores, therefore, serve as the inoculum source when YST seedlings germinate in the winter. Approximately six urediniospore generations can be expected to occur each year in central-northern California under suitable conditions for disease.
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1. Introduction

Yellow starthistle (YST, *Centaurea solstitialis* L.) is an exotic weed of Eurasian origin that infests rangeland and natural areas in the western United States and Canada. In California alone, YST has invaded over seven million ha and continues to spread (Pitcairn et al., 2006). Yellow starthistle is a winter annual adapted to the mild, wet winters and hot, dry summers typical of a Mediterranean climate. In California, YST seeds begin to germinate in the fall (October to December) with the start of the rainy season, rosettes develop through the winter (December to March), plants bolt and flower from late spring into summer (June to September), and senesce during the hot, dry weather of the late summer and fall (Maddox, 1981).

The rust fungus *Puccinia jaceae* Otth var. *solstitialis* (*P. j. solstitialis*) is macrocyclic and autoecious. The distribution of *P. j. solstitialis* is similar to that of the native range of YST in southern Europe and western Asia (Savile, 1970). *Puccinia jaceae* var. *solstitialis* was introduced into California in 2003 for biological control of YST (Woods and Villegas, 2004). At some release sites, multiple generations of urediniospores have been observed in California during the spring and summer, when conditions of temper-

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ature and moisture were favorable for plant growth and disease (Fisher et al., 2007). Teliospores develop in the summer as weather becomes hot and dry and plants senesce (A. Fisher and D. Woods, personal observation). Teliospores are functional, i.e., they germinate, although infection of YST by teliospores was not shown in the greenhouse (Bruckart and Eskandari, 2002). Based on the general behavior of this fungal species, *P. jaceae* teliospores germinate in the winter to produce basidiospores, which infect plants and give rise to pycnia (Savile, 1970). Indeed, part of this life cycle was confirmed when pycnia were observed in the winter at a site that had been inoculated the previous spring (Fisher et al., 2006).

Currently, *P. j. solstitialis* has been introduced to multiple YST populations in CA that represent extremes of habitat and climate (Woods and Villegas, 2006). This has been possible because of a program to produce urediniospores in greenhouses. However, the process of cultivating, harvesting, and preserving urediniospores is labor intensive; there is no artificial medium for the production of this rust, so propagation of spores must be conducted on plants. It requires inoculating large numbers of plants in a greenhouse, vacuum-harvesting urediniospores, and freezing inoculum at −70 °C until use in field inoculations (Woods and Popescu, 2004). Thus far, this has been the only source of inoculum for establishment of new release sites. However, if infected leaves from the field could be used as an inoculum, this could greatly reduce costs.

Information about the field performance of urediniospores, including length of viability, their possible role as a source of initial inoculum for the next growing season, and latent period (the time it takes for pustules to erupt) is necessary to determine the potential usefulness of field-grown urediniospores for artificial field inoculations of YST. Field-grown biological control agents are often used to facilitate establishment in new locations. Examples are the insect agents *Metzneria paucipunctella* Zeller, *Bangasternus fausti* Reitter, *Terellia virens* (Loew), *Urophora affinis* Frfld. and *Urophora quadrifasciata* (Meigen) on spotted knapweed (*Centaurea stoebe* L. [= *C. maculosa* Lam.]) (Wilson and Randall, 2003), the gall mite *Aceria malherbae* Nuzzaci on field bindweed (*Convolvulus arvensis* L.) (McClay et al., 1999), the gall nematode *Subanguina picridis* (Kirj.) Brezeski. on Russian knapweed (*Acroptilon repens* L.) (Story et al., 2004), and the dyer's woad rust fungus (*Puccinia thlaspeos* C. Schub.) for biological control of dyer′s woad (*Isatis tinctoria* L.) (Kropp et al., 2002). If *P. j. solstitialis* urediniospores are sufficiently stable in the field, then naturally-infected YST may serve as a source of inoculum available to local weed managers, thus enabling them to make independent decisions regarding distribution to new inoculation sites. Furthermore, urediniospores may play a role in survival of *P. j. solstitialis* between seasons, knowledge of which will further the independent use of this fungus for biological control of YST.

In addition to the inoculum source, population growth of *P. j. solstitialis* will be limited by the number of urediniospore generations each year. At constant temperatures, pustules erupted within 15 days of inoculation at 15 °C and within 10 days after inoculation at 20 or 25 °C (Bennett et al., 1991). Temperatures in the field, however, vary constantly, and data about latent period in the field is needed to understand urediniospore performance under natural conditions.

The goals of this study were to measure *P. j. solstitialis* urediniospore viability in the laboratory and field and to measure the latent period under controlled temperature regimes representative of field conditions. Experimental latent period data was then compared to observed latent periods in the field. Using these data it is possible to evaluate the potential usefulness of field-grown urediniospores for artificial deployment of *P. j. solstitialis* in California, to determine whether urediniospores have a role in persistence of *P. j. solstitialis* between seasons, and to estimate the potential number of urediniospore generations each year.

2. Materials and methods

2.1. Inoculum source

Puccinia jaceae var. *solstitialis* field isolate FDWSRU 84-71 was collected in 1984 east of Yarhisar and Hafik (Sivas), Turkey by Sarah Rosenthal (USDA, Retired). From 1984 to 2003 this isolate was evaluated in quarantine at the USDA, ARS, Foreign Disease-Weed Science Research Unit (FDWSRU), at Ft. Detrick, MD. With approval from the USDA, Animal & Plant Health Inspection Service (APHIS), inoculum was moved from the containment greenhouse at FDWSRU to the California Department of Food and Agriculture (CDFA). Urediniospores were propagated at CDFA, in Sacramento, CA. To propagate spores, potted YST plants were inoculated with a urediniospore suspension, exposed to dew without light for 16 h at 20 °C, and incubated in a 27 °C greenhouse with natural light for 14 days, until pustules formed. Spores were harvested using a vacuum spore collector (Cherry and Peet, 1966) and stored at −70 °C.

2.2. Urediniospore viability after dry storage in the laboratory

Urediniospore viability was evaluated *in situ* on intact, dry YST leaves. Seven 10–12 week old potted YST rosettes were spray-inoculated, using plastic 250 ml finger pump spray bottles, with a suspension of 100 mg *P. j. solstitialis* urediniospores from CDFA in 200 ml water and 0.15% Tween® 20 (polyoxyethylene sorbitan monolaurate; EM Science, Gibbstown, NJ). Plants were placed in a dew chamber overnight as described above and then observed for symptoms in the CDFA greenhouse. When pustules became visible, infected leaves were removed and the leaves from each plant were placed in separate brown paper bags (7 bags total). To ensure consistency between bags, samples were weighed and adjusted to a uniform biomass before

placing them on a laboratory bench to dry at room temperature, approximately 22 °C, at Western Regional Research Center (WRRC), Albany, CA. At the time of leaf removal and every seven days thereafter until viability was zero, spores from one leaf per bag were scraped into 0.1% water agar (WA) with 0.15% Tween® 20 (WAWA; Bruckart, 1999). Three 10-μl drops of suspension were plated on 1.5% WA in 10 cm diameter petri plates. Plates were incubated at 20 °C in the dark. The percentage of germinated spores ($n = 100$) in each drop was recorded after 24 h.

After 13 weeks, spores from 10 mg of infected plant tissue per bag were exposed to high humidity to determine if hydration would increase spore germination. Leaves were placed in an open 1.5-ml vial within a sealed container with water at the bottom and incubated at 20 °C for seven days. Relative humidity inside the sealed container was 99% according to a digital thermohygrometer (Control Company, Friendswood, TX). Viability was assessed for these samples as described above. The experiment was repeated three times.

The Weibull function, commonly used to model survivorship processes (Gross and Clark, 1975), was used to estimate rate of loss in spore viability on dry leaves using nonlinear regression (Statistica® release 5, SatSoft Inc., 1997). Viability data both at harvest and at week 13 were omitted from the regression analysis because urediniospores were either fresh or had been hydrated, thus constituting different treatments.

2.3. Urediniospore viability in the field

Forty 5 week old, greenhouse grown YST plants were transplanted into a field plot at CDFA in January 2005. Plants were inoculated, covered overnight with a plastic tent enclosure, and then monitored weekly. First pustules appeared in February and several generations of urediniospores were observed during the growing season. Infected leaves and stems remaining on plants through the spring and summer were collected in September 2005. Infected leaves and stems were distributed between 88 5×8-cm, white, 100% polyester fabric bags tied shut with 100% polyester twine. Half of the bags were taken to the WRRC, Albany, CA (in the San Francisco Bay Area), and half were brought to Armstrong Field Station, University of California, Davis (in the Central Valley), on the day plants were harvested. At each site, bags were placed on the ground in a wire mesh basket attached to a fence post. From September 28, 2005 to May 11, 2006, four bags were collected every three weeks from each site and brought to the WRRC to assess spore viability.

A variant of this experiment was conducted during 2006 to 2007. In the second year of the experiment, infected leaves were harvested from inoculated greenhouse-grown plants and urediniospores were harvested and tested immediately for spore viability. These data were used as the standard for comparing germination of subsequent field samples, thus enabling a determination of loss in spore viability over time. Forty potted greenhouse-grown YST plants were inoculated at CDFA as described for 2005, and returned to the greenhouse. Infected leaves were harvested after two to three weeks when pustules had fully erupted. Bags were assembled as described above and returned to the field. Tests for spore viability were made every three weeks from June 22, 2006 to February 7, 2007.

Spore viability was assessed for each bag as before and the percentage of spores germinated ($n = 100$) in each drop was recorded after 24 h. The Weibull function was used to estimate the rate of loss of spore viability. The Proc Mixed procedure in SAS (SAS Institute software version 9.1) was used to determine if spore viability differed between samples brought to Albany and Davis. Location (either Albany or Davis) was included as a fixed factor and bag and drop on the petri plate were random factors.

2.4. Latent period in the laboratory

To determine the effect of day and night temperatures on latent period in the laboratory, one- to five-week-old rosettes were inoculated with 50 mg of *P. j. solstitialis* urediniospores suspended in 100 ml Tween-water in the CDFA greenhouse. Plants were placed in a commercial dew chamber for 16 h at 20 °C. Then, rosettes in each age class were evenly divided into three groups and placed in environmental chambers (Percival Scientific, Inc.) representing three temperature regimes. Chambers (each with a 12-h photoperiod) were set to mimic day and night temperatures for winter (=2 °C dark/12 °C light), spring (=5 °C dark/15 °C light), and early summer (=12 °C dark/20 °C light) in central California. Plants were observed every two to four days after inoculation and the percentage of infected plants was recorded. The experiment was repeated three times.

2.5. Latent period in the field

To determine actual latent periods in the field, permanent experimental plots were established in January 2005 and 2006 in the coastal hills outside the city of Napa, California, and in the Central Valley near Woodland, CA. The Napa site is in a valley in the coastal hills at 427 m elevation, dominated by YST, European annual grasses, native grasses and shrubs, and surrounded by oak woodland. The Woodland site is a rangeland at 53 m elevation in the Sacramento Valley, dominated by European annual grasses and herbs, and surrounded by actively grazed range and agricultural land.

Six blocks, each containing five permanent 1×0.5 m plots, were established at each site in 2005. In 2006, plots were smaller, 0.5×0.5 m, to conserve spores. Within each block, one plot was inoculated in January, one in February, one in April, one in May, and one in June. Plots were inoculated on the same dates in 2005 and 2006 with 50 mg spores suspended in 200 ml Tween-water. Plots were observed every seven days and scored as either positive

or negative for disease symptoms. SAS Institute software was used to determine if the time for pustules to erupt differed between the Napa and Woodland field sites. Avatel™ weather stations (Elucit, Inc., Mendocino, CA) and Hobo Pro Series data loggers (Onset, Bourne, MA) were maintained at both sites to collect temperature data. Mean temperature data after the April, May and June 2005 inoculations in Napa were estimated due to a weather station outage.

To estimate the number of asexual urediniospore generations that occur per year, it is necessary to identify the beginning and end of the season when urediniospores are infecting YST. To determine when the first urediniospore pustules erupted in the early spring, release plots at the Woodland site, and a plot of previously inoculated YST at CDFA in Sacramento, were visually inspected for urediniospores beginning in the winter of 2005 and 2006. Plant senescence at the Woodland site was used to estimate the end of the season for urediniospore activity.

3. Results

3.1. Urediniospore viability after dry storage in the laboratory

There was no difference in viability between replicates ($P = 0.8176$), and therefore data were pooled. Viability decreased from 33.6% to 19% during the first week after infected plant material was harvested and stored in the laboratory at room temperature (Fig. 1). After the first week, viability decreased gradually to less than 10% by week six and to less than 1% by week 10. Viability was fit by the Weibull equation ($\%\ \text{Germination} = C * \exp(-((X/\lambda)^{k}))$), where X was the independent variable (weeks since harvest of urediniospores), λ is the shape parameter, k is the scale parameter, and C is the maximum germination rate. Parameter estimates were: $\lambda = 6.716 \pm 0.428$ (SE), $k = 3.018 \pm 1.148$, $C = 18.00 \pm 1.76$ ($P < 0.00001$ for λ and C, and $P < 0.013$ for k, $R^2 = .87$). The fact that $k > 1$ indicates that the rate of survivorship decreases as the spores age. At week 13, hydrating urediniospores at high humidity for one week increased germination rates to approximately 5%.

3.2. Urediniospore viability in the field

Urediniospore viability from each sampling date from September 2005 to May 2006 was very low, ranging from 0% to 0.2% (data not shown) for bagged samples placed in the field. Viability was not tested immediately after spores were collected in 2005. In 2006, germination rates were high immediately after infected leaves were collected in June 2006 (78.5% ± 2.6 SE), but dropped to 10.7% (±2.8 SE) in Albany and 2.1% (±0.8 SE) in Davis after three weeks in the field. After six weeks, viability was 2% at Albany and 0% at Davis, and germination was negligible (<0.1%) at both sites during each sampling date thereafter (Fig. 2). Data on viability was fit by the Weibull function. Parameter estimates were: $\lambda = 2.972 \pm 0.003$ (SE), $k = 73.0 \pm 9.4$, $C = 78.5 \pm 2.0$ for Albany ($P < 0.0001$ for λ, k and C; $R^2 = .98$), and $\lambda = 0.826 \pm 0.121$, $k = 1$ [parameter omitted], $C = 78.5 \pm 1.26$ for Davis ($P < 0.0001$ for λ and k; $R^2 = .99$). Parameters λ and k were significantly different for the two sites; however, viability differed only at weeks three ($P = 0.0018$) and six ($P = 0.0120$).

3.3. Latent period in the laboratory

As environmental chamber temperatures increased, the time it took for pustules to erupt (latent period) decreased (Fig. 3). At simulated winter temperatures (2/12 °C), pustules first erupted 24 days after inoculation, and the percentage of plants with pustules continued to increase until day 32. At simulated early spring temperatures (5/15 °C), pustules first erupted 17 days after inoculation

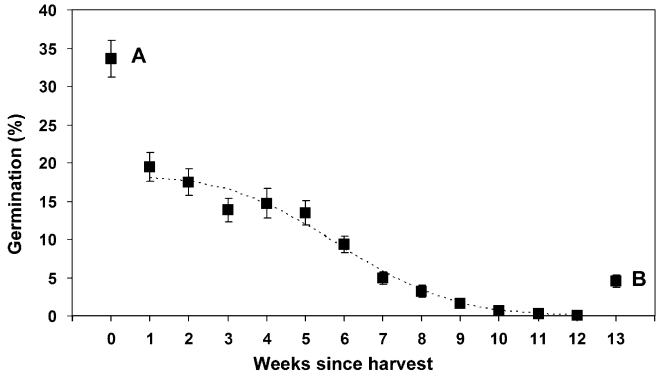

Fig. 1. *Puccinia jaceae* var. *solstitialis* urediniospore viability after dry storage at room temperature (means ± SE). Data from three replicates presented for each sampling date. The data point to the left of the letter A was collected immediately after harvest and the data point to the left of the letter B was collected after spore hydration. Curves generated by the Weibull analysis.

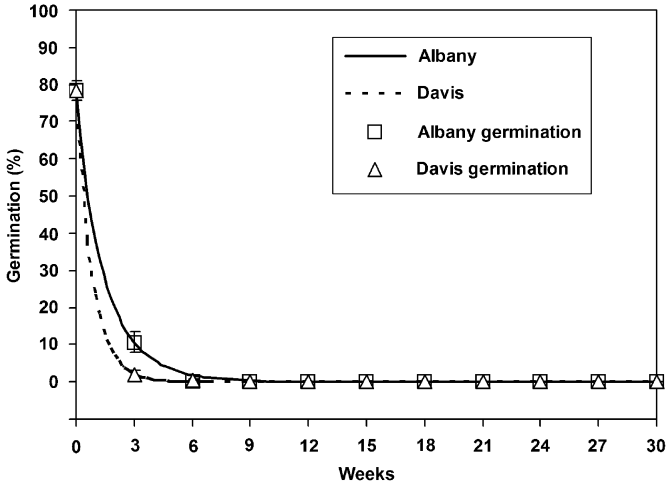

Fig. 2. *Puccinia jaceae* var. *solstitialis* urediniospore viability (means ± SE) in Albany and Davis, CA, from June 2006 to February 2007. Curves generated by the Weibull analysis.

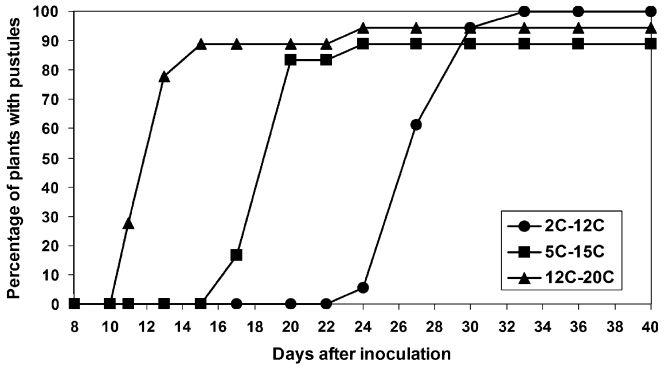

Fig. 3. Mean number of days after inoculation with *Puccinia jaceae* var. *solstitialis* until pustule eruption following plant incubation at three temperature regimes (night/day, °C) simulating winter (2/12), spring (5/15), and summer (12/20) for central California.

and pustules continued to increase until day 24. At early summer temperatures (12/20 °C), the first pustules erupted 11 days after inoculation and the percentage of plants with pustules continued to increase until day 15 (Fig. 3). Regardless of how long it took for pustules to erupt, the final percentage of plants with pustules was about the same (90–100%).

3.4. Latent period in the field

At both field sites, the length of time for pustules to erupt decreased as temperatures increased from winter to summer, which was similar to results in the laboratory. Latent period ranged from four to five weeks in winter to one to two weeks in spring (Fig. 4A and B). In Woodland, in 2006, temperatures averaged 15 °C max. and 6 °C min. after the February inoculations. This resulted in slower pustule development than after the January inoculations when temperatures averaged 17 °C max. and 7 °C min. Pustules erupted more slowly in Napa than Woodland ($P < 0.0001$) in both years, likely because of the relatively cooler temperatures at the Napa site, especially during May and June (data not shown). The relationship between latent period (Y) and temperature (X) was described by the equation: $Y = 6.927 + (-0.299)X + (0.005)X^2$ ($R^2 = 0.87$) in Napa and $Y = 7.610 + (-0.540)X + (0.012)X^2$ ($R^2 = 0.80$) in Woodland, CA (Fig. 5).

The beginning and end of the season when urediniospores infect YST was monitored in the Central Valley in 2006. The first uredinia were observed in Woodland and at CDFA in Sacramento on February 15 and 21, respectively. Yellow starthistle at both sites began to senesce in August 2005 and in June 2006.

4. Discussion

Redistribution of rust fungi, via infected plant material, can be an efficient method of establishing new populations for biological control of weeds (Kropp et al., 2002). In the *P. j. solstitialis*—YST pathosystem, the pathogen could be

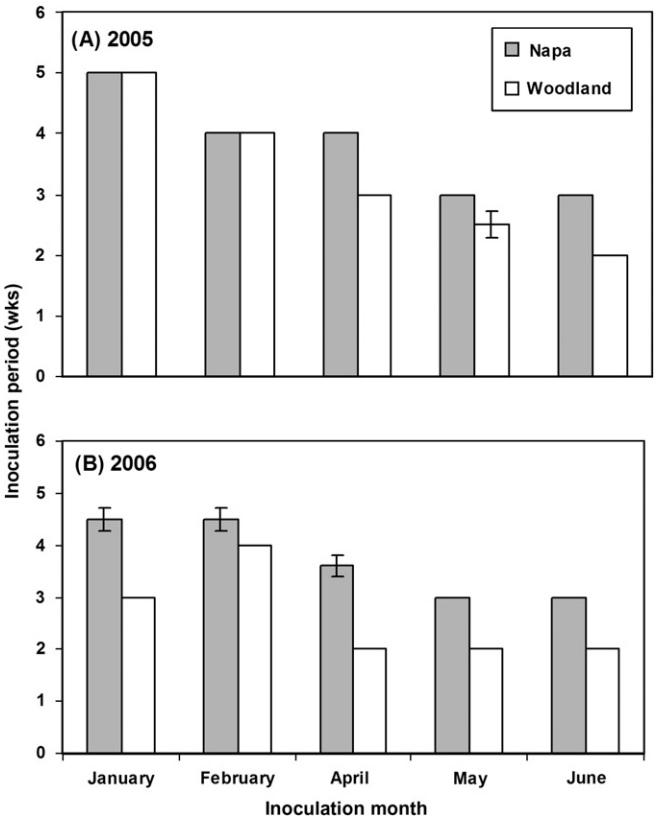

Fig. 4. Incubation period (time to pustule eruption) on yellow starthistle inoculated with *Puccinia jaceae* var. *solstitialis* in: (A) 2005 and (B) 2006 in Napa and Woodland, CA (means ± SE). If standard error bars are not present, pustules erupted in all plots during the same week.

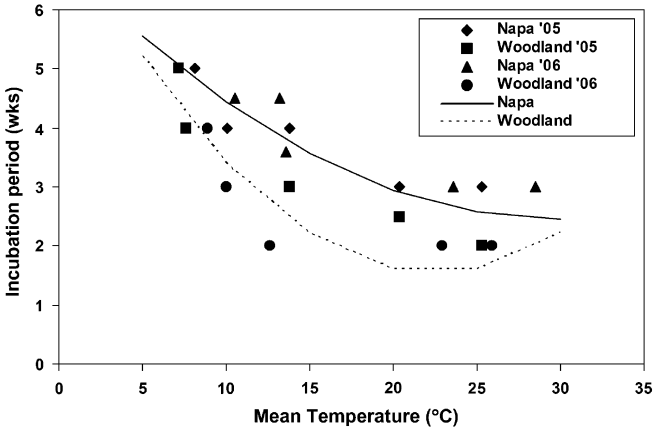

Fig. 5. Regression of *Puccinia jaceae* var. *solstitialis* incubation period (Y, time to pustule eruption) on field temperature (X) described by $Y = 6.927 + (-0.299)X + (0.005)X^2$ ($R^2 = 0.87$) in Napa and $Y = 7.610 + (-0.540)X + (0.012)X^2$ ($R^2 = 0.80$) in Woodland, CA.

redistributed either as urediniospores or teliospores. Teliospores are produced in the late summer during plant senescence (D. Woods, A. Fisher, personal observation) and therefore redistribution could only occur after the YST growing season is over. Urediniospores multiply asexually by infecting new plants or plant parts. If urediniospores were redistributed to new sites, successful infection

could be evaluated in two to five weeks depending on ambient temperatures. To successfully redistribute urediniospore infected plant material to new sites, urediniospores must remain viable until conditions are suitable for infection. In our study, *P. j. solstitialis* urediniospores had a relatively short life span both in the laboratory and field at ambient temperatures. This suggests redistribution of the pathogen would have to be done immediately after pustules emerged for there to be any potential for success.

Harvesting leaves infected with *P. j. solstitialis* urediniospores and storing them dry at room temperature indoors led to a decrease in viability over a period of 10 weeks, at which time germination was essentially zero. These data are comparable to those of urediniospores of *Puccinia glumarum* (Schm.) Eriks. & Henn., which lost viability over 58 days when stored at room temperature (Hungerford, 1923) and over 88 days when stored between 9 and 13 °C (Raeder and Bever, 1931). In the first week after harvest, *P. j. solstitialis* germination dropped 42%. Low urediniospore viability even after one week on dry plant material in the laboratory eliminates this option of long-term storage of inoculum. After storage in the laboratory, germinations decreased 58% after three weeks and 74% after six weeks. Because viability declines quickly over time, field-grown inoculum for new releases would have to be used soon after harvest, and pustules would not provide high quality inoculum for longer than a few weeks. Hydration of dried *P. j. solstitialis* urediniospores after storage resulted in an improvement, however germination rates were still low, approximately 5%.

In a comparison among methods to establish the dyer′s woad rust, leaves infected with teliosori were collected in the spring, then dried and ground into fragments. Dried plant material was either sprinkled on plants or placed on the soil next to plants in June, and these inoculations resulted in disease symptoms (Kropp et al., 2002). There are many differences between the dyer′s woad rust and *P. j. solstitialis*. For example, *P. thlaspeos* is microcyclic and produces a systemic infection. However, in both biological control systems there is a need to develop scaleable methods to establish the pathogen in new weed populations. The short survival time of *P. j. solstitialis* urediniospores likely will limit use of redistributing dried infected plant material or any similar approach for biological control of YST.

Based on results obtained in this study, urediniospores will not successfully serve as a source of primary inoculum when YST seedlings emerge. In the field, between 0% and 0.2% of spring produced urediniospores remained viable through the summer dry season in the first year of the experiment, and viability was zero during this time in year two. In a study investigating release strategies, *P. j. solstitialis* urediniospores were released near Woodland in the spring of 2005 and rust infections were noted on YST the following year (spring 2006) (Fisher et al., 2007). The source of inoculum in the winter is presumed to be teliospores; viability of teliospores has been demonstrated in the laboratory (Bruckart and Eskandari, 2002), and pycnia were observed at the Woodland release site (Fisher et al., 2006).

Understanding the mechanisms of survival during times of unfavorable weather is important for identifying inoculum sources and the potential for seasonal epidemics in rust fungi. In a classic example, urediniospores and dormant mycelia of wheat leaf and stem rusts caused by *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* and *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks & E. Henn. overwinter on site or urediniospores are transported from overwintering areas to serve as principal inoculum sources. Similar to *P. j. solstitialis*, during the summer, when maximum temperatures were above 30 °C and minimum temperatures above 10 °C, 60% of urediniospores of both species survived five days and germination was negligible after 19 days (Eversmeyer and Kramer, 1994). The rust fungus *Puccinia lagenophorae* Cooke, proposed as a biological control for *Senecio vulgaris* L., survives in a living host over winter and therefore could serve as an inoculum source in the spring (Frantzen and Müller-Schärer, 1999). Yellow starthistle is a winter annual, however, and if moisture is available, YST can remain green all year in CA (L. Smith, personal observation). There is a possibility that *P. j. solstitialis* urediniospores retain viability longer on living plant tissue, and rare YST populations retaining green tissue year round could be a source of urediniospores in the spring. This hypothesis was not tested in the present study. Results from this study indicate that *P. j. solstitialis* urediniospores are not likely to survive the summer on dry YST, and they are not likely viable by the time winter arrives, even in cool-moist climates similar to coastal California, as represented by the Albany site. Based on this, they are not a likely source of primary inoculum when YST germinates in the winter. In the absence of living plant tissue, survival of *P. j. solstitialis* in California depends on teliospores as a source of primary inoculum, and population increase depends on multiple generations of urediniospores during the YST growing season.

When urediniospores were harvested immediately after pustules erupted, bagged and returned to the field, viability decreased more quickly than when they were stored in the lab. After three weeks, there was a 58% loss of viability in the laboratory compared to an 86% reduction at the Albany site and a 97% reduction in Davis. *P. jaceae* is a species morphologically adapted to semi-arid Mediterranean climates. Adaptations to avoid desiccation include wall pigmentation, increased spore size, and increased wall thickness (Savile, 1970). Even with these characteristics, urediniospores stored outside lost viability over a period of six weeks at the Albany site, which has relatively cool summer temperatures and intermittent clouds and free moisture in the form of fog from the San Francisco Bay. In addition, spore germination did not increase in the field when bags were evaluated during the winter in both years, suggesting that natural conditioning factors, including hydration in the form of dew or rain, do not affect or improve urediniospore germination. If viability was low

at the Albany site, with mild summer climate conditions, it is unlikely there are other sites in California where urediniospores survive longer.

Latent period for the development of pustules after inoculation by *P. j. solstitialis* urediniospores generally decreased from winter to summer as average temperatures increased. The minimum time for pustules to erupt was 11 days at 12/20 °C in the environmental chamber and the maximum was 32 days at 2/12 °C. Latent period in the field ranged from one to two weeks to four to five weeks. Our field results are similar to what would be predicted using the regression equation described in Bennett et al. (1991), which was based on constant temperature experiments. If other climatic conditions, such as available moisture, are suitable for urediniospore infections, and we assume a five week latent period in February, four weeks in March, three weeks in April and May, and two weeks in June, a conservative estimate would be six urediniospore generations could occur per year.

Latent period data for *P. j. solstitialis* was similar to that of both *Puccinia chondrillina* Bubak & Syd., released for the biological control of rush skeleton weed, *Chondrilla juncea* L. (Emge et al., 1981) and *Puccinia carduorum* Jacky released for the biological control of musk thistle, *Carduus nutans* L. (='*thoermeri*') (Politis and Bruckart, 1986). For each pathosystem, there was a low level of infection at cool temperatures (~10 °C), optimal infection between 18 and 21 °C, and no infection above 24–25 °C. Since its intentional introduction, *P. chondrillina* became established in the United States and has been considered successful in control of rush skeleton weed in California (Supkoff et al., 1988). *P. carduorum* became established in Virginia where it hastened plant senescence and reduced seed production (Baudoin et al., 1993; Kok, 2001). It has since naturally dispersed to California (Woods et al., 2002). Results from our study suggest that *P. j. solstitialis* uredinia occur only during the period of active YST growth, so permanent establishment will be limited to areas where conditions are suitable for all *P. j. solstitialis* spore stages.

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